NSG-464

SEMI-ANNUAL PROGRESS REPORT

ON

GRANT NSG-464

SUBMITTED TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

FOR THE PERIOD

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to

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INTRODUCTION

Those results obtained for the first three semi-annual periods (April 1, 1963-November 1, 1964) have been detailed in previous reports. This report deals only with those results obtained during the period, November 1, 1964 to April 1, 1965. Results obtained by Dr. Cooney in his investigations on thermopholic fungi during this period will be covered in a separate report to be submitted by him.

I. Studies on Avena sativa

A major portion of the research effort during the period covered by this report has been devoted to a continuation of the previous studies on Avena sativa. These investigations have comprised both physiological and ultrastructural studies in which the primary goal has been the determination of those biochemical and biophysical events leading to cytomembrane rupture in plants which have been stressed by exposure to extreme low pressures. A basic assumption in all of this work, justified by the results obtained and reported upon in the previous semi-annual reports, has been that the anaerobic environment, rather than low pressure, per se, is the factor primarily responsible for the deleterious results observed.

A. Photoperiod Studies

As indicated in the previous semi-annual reports, observations during the spring and summer of 1964 demonstrated that the "lethal" exposure time to extreme low pressure (v.p. level of H_2O at 20°C, -17 mm. Hg.) for young oat seedlings increased from a minimum of approximately 6 hours to a maximum of 18-1/2 hours during the summer. Following this peak, the lethal time decreased again to a low of 6 hours during the winter of 1964-65.

Growth chamber experiments were programmed to isolate the environmental factors responsible for this behavior. Seeds of Avena sativa var. "Bonkee" were planted in soil in clay pots and placed in vermiculite packed trays. These were placed in a Percival growth chamber, Model PGC-78 in which germination and growth subsequently occurred. Light provided by the fluorescent and incandescent light bank ranged from 2000 ± 100 foot-candles at the top of the pots to 2400 ± 100 foot-candles at the tip of the seedling leaves. The plants were grown for 16-18 days in all of the programmed cycles prior to testing.

(A) Short Day, Warm Temperature Cycle

The chamber was programmed for a 10 hr. light and 14 hr. dark cycle. Dry bulb temperature during the daily cycle was controlled to range between 86°F. and 66°F., while wet bulb temperatures during the same interval ranged from 62°F. to 80°F. Seedlings grown under this cycle survived up to 6 hours of exposure to near vacuum with no evident macroscopic effects. Plants exposed for 8, 10, 11, and 12 hours showed discoloration of some of the leaves, however, at least 3/4ths of the plants in each pot (11± 1 plants) remained alive. Plants exposed to 14, 16, 17, 18 and 20 hours generally were killed; however, in some pots exposed for 15 and 17 hours, some plants, but generally less than 3/4ths remained alive.

(B) Short Day, Warm Temperature Cycle, - Interrupted Nyctoperiod

A second cycle was programmed with the same sequence of light and temperature cycles as the preceding; however, the nyctoperiod was interrupted in the middle by the illumination provided by a 60 watt

incandescent bulb for a 15 minute period. Under these conditions, seedlings exposed for 8, 9, 10, 11, and 12 hours showed some discoloration, but at least 3/4ths of the seedlings in each pot survived. The shortest lethal time in which all of the seedlings in an individual pot died appeared to be 14 hr., while other plants exposed for 13, 15 and even 19 hrs. showed some survival although in each of these cases less than 3/4ths of the plants in each pot survived. The differences in survival between the plants grown under the two similar cycles with the single difference of an interrupted nyctoperiod do not appear to be significant.

(C) Long Day, Warm Temperature Cycle

A third cycle was programmed in which the photoperiod consisted of 16 hrs. of light alternating with 8 hrs. of dark. The dry bulb temperature ranged between 86°F. and 66°F. The temperature remained above the 80°F. mark for a total of 9 hrs. while in the previous two cycles it was above this point for only 6-3/4 hrs. The lethal exposure time following this cycle was as low as 10 and 11-1/2 hrs. However, other pots of seedlings survived 7,10,11,12,13 and 14 hours of stress with some discoloration and at least 3/4ths of the plants remained viable. Other pots exposed to 12,15, and 17 hrs. of low pressure showed more macroscopic damage and less than 3/4ths of the plants in each pot survived.

(D) Short Day, Warm Temperature Cycle

A short photoperiod cycle of 10 hr. light and 14 hr. dark was programmed in which the temperature was caused to rise above 80°F. during the last

1-1/2 hr. of the nyctoperiod and maintained above this point for a total of 11-3/4 hrs. Under these conditions pots exposed for 12, 15 and 16 hrs. to low pressure showed extensive discoloration and death of all the seedlings. Other pots exposed for 7,8,10,10-10½,11 and 17 hours showed less damage with, however, less than 3/4ths of the seedlings surviving. Others exposed for 6, 13 and 14 hours showed still less damage with more than 3/4ths of the seedlings in each pot surviving.

(E) Short Day. Low Temperature Cycle

A short photoperiod cycle of 10 hr. light and 14 hr. dark was programmed with a temperature cycle of 75°F. to 56°F. Under these conditions the lethal period of exposure for all of the seedlings in a single pot was reduced to 6 hrs. Pots of seedlings exposed to 7,8,9 and 11 hrs. also were severely damaged and in only one instance at seven hours did any seedlings survive. Pots exposed for 2,3,4,4-1/2 and 5 hrs. showed some damage although in each instance more than 3/4ths of the seedlings survived the stress successfully.

Figures 1 and 2 summarize the results obtained from these 5 cycles. In conclusion, it appears that survival time is independent of the photoperiod, but is strongly dependent on the temperature cycle imposed on the plant during growth. Threshold "lethal" time for all of the seedlings in a single pot was shortened from 14 hrs. to 6 hrs. as a consequence of growth under a temperature regimen approximately 10°F. cooler.

B. Experiments with Decenylsuccinic Acid

The application of certain unsaturated fatty acids to plants is known to alter cellular permeability and dependent physiological phenomena. KUiper (1964) found that the application of decenylsuccinic acid increased drought resistance in beans and increased frost resistance in pears and apples. Increased drought resistance presumably resulted from a greater degree of permeability of cellular membranes. As indicated in earlier reports in the present study on oats certain respiratory products are assumed to accumulate to toxic concentrations within cellular membranes. Consequently an experiment was designed to alter cellular permeability by the application of decenylsuccinic acid in an attempt to determine whether a permeability change would affect ultrastructural membrane deterioration under reduced pressure conditions. Application of the decenylsuccinic acid was carried out in two ways, either by intensive spraying immediately prior to exposure to low pressure or by watering with a dilute solution of decenylsuccinic acid ($3X10^{-3}M$) over the 16 day growth period of the seedlings. The experimental group which has been treated with the acid by spraying showed no significant difference in survival time under low pressure. Those seedlings which had been intermittently watered with the acid, however, showed a slight increase in ability to resist low pressure effects. Results were not always consistent and it will not be possible to conclude that DSA affects survival time until additional replications of the experiment have been carried out.

C. Mineral Deficiency Tests

Preliminary tests with oat seedlings grown in nutrient deficient soils led to the speculation that perhaps certain element deficiencies adversely affected the survival of plants under low pressure. In addition, it is

known that in the absence of inorganic phosphate, fermentation stops at the level of glyceraldehyde-3-phosphate (Harden and Young, 1908).

Consequently, hydroponic experiments were set up in which oats were allowed to germinate and grow for 1 week in vermiculite and then transferred to various hydroponic solutions with Hoagland's complete medium as the standard. Experimental groups lacking sulfur, phosphorous, or nitrogen were exposed to low pressure for varying times along with controls grown in complete media.

Fig. 3 shows a series of seedlings which had been grown in a complete medium at a time 15 days after exposure to low pressure. A graded response is apparent which is dependent on the period of exposure to low pressure. Fig. 4 illustrates the response of seedlings grown in a -N medium at a time 22 days after exposure to low pressure. It is particularly apparent here that the degree of damage inflicted by exposure to low pressure is much less severe especially with regard to the 7 and 8 hr. groups. Fig. 5 depicts the response of a series of seedlings grown in a -P medium at a time 43 days after exposure to low pressure. The -P plants grew only feebly, nevertheless it is apparent that the plants exposed to 6 and 8 hrs. of near vacuum did manage to survive and continue growth nearly as well as the -P control. Fig. 6 shows the response of a series of seedlings grown in a -S medium at a time 32 days after exposure to low pressure. The -S plants exposed to 5 and 7 hrs. of near vacuum appear to have made better growth than the -S control. Correlated with these observations, Fig. 7 shows the response of 4 groups of seedlings grown in complete, -S, -P, and -N media and exposed to near vacuum for 5 1/2 hrs. Here the -S vacuum-stressed plants appear to be as vigorous as the controls grown in complete media. This response of the -S plants is considerably enigmatic and replications of this experiment are planned to verify the validity of these observations.

Although the results at this point are far from conclusive, it does appear that major element deficiencies will alter the survival time of seedlings exposed to low pressure and perhaps in certain cases confer additional resistance to

stress conditions.

D. Root Removal Tests

Betz (1958a)and Ramshorn (1957) have reported that under conditions of oxygen deficiency a shunting of pyruvate away from the tricarboxylic cycle to an acetaldehyde -alcohol pathway occurs. Barker and el Saifi (1952) showed that in potato tubers placed under anaerobic conditions lactic acid formation rapidly increased, followed by a decrease after 7 days, and a subsequent increase in alcohol production. Keneflick (1960) showed that while certain plants were capable of adapting to flooding, a submergence of the whole plant resulted in the accumulation of alcohol. Grineva (1960,61,62) and Betz (1957, 1958b) have reported that actively growing root meristems accumulate ethanol.

Inasmuch as the macroscopic pattern of damage observed in the present series of experiments on oat seedlings appears to be partially the result of a transport phenomenon and since the literature indicates that alcohol and perhaps other toxic materials may be expected to accumulate in roots under anaerobic conditions, a series of experiments were designed in which it was proposed to test the effect of root removal on survival time of oat seedlings under near vacuum . Oat seedlings after germination were supported with their roots immersed in Hoagland's solution. These were divided into three groups. One group had the roots removed, a second group had ethyl alcohol (200 mg. %) added to the solution, while the third group served as a control. The three groups were then exposed for a threshold lethal time to near vacuum. The groups grown in the solution to which alcohol had been added showed the most severe damage on exposure to reduced pressure. The group of seedlings, the roots of which had been removed, showed approximately the same extent of damage as the control group. However, when compared to

controls, the roots of which had been removed, but which were not subjected to low pressure, it was apparent that the effect of root removal was deleterious to subsequent growth until a new set of roots had been formed. In the light of this observation it appears that the harmful effect of root removal on the experimental group may be just offset by the removal of a source of atoxic substance formed in the roots during anaerobiosis which would otherwise be transported to the tops of the plants. However, additional experiments on a species of plant less refactory to root removal will be needed to affirm this conclusion.

E. Alcohol Determinations

Various studies (Phillips, 1947; Vlamis and Davis, 1943) have indicated that among the cereal crops, rice has the strongest fermentation system. App and Meiss (1958) have shown that ethanol is an efficient inducer of alcohol dehydrogenase in rice plants. It would appear that a strong positive feedback system for the production of alcohol dehydrogenase exists in rice with alcohol serving the dual role of a terminal metabolite as well as a "trigger" molecule which stimulates increased alcohol dehydrogenase production. Hageman and Flesher (1960) working with corn seedlings showed on the other hand that ethanol decreased alcohol dehydrogenase activity. One could, perhaps, compare this to a negative feedback system.

Earlier reports on this research project have speculated that alcoholic fermentation may be the factor responsible for membrane degradation within the cells of oat leaves. In order to determine whether alcohol in fact accumulated in deleterious amounts in the leaves of low pressure stressed plants, spectrophotometric tests were carried out. Tissues to be analyzed

were homogenized and the diluted slurry placed in the diffusion chamber of Conway cells (with Obrink modification). A microdiffusion technique (Kaye, 1963) with a potassium dichromate-sulphuric acid detection reagent was used. Measurements after incubation were made with microcells in a Beckman DU spectrophotometer at 450 millimicrons. A calibration curve was constructed with known amounts of ethanol in water ranging from 250 mg. % to 6.25 mg. %. The controls consisted of tissue from plants not exposed to low pressure, control tissue to which a known amount of ethanol was added before homogenization, and control tissue to which a known amount of ethanol was added after homogenization. The concentration of alcohol averaged 12 mg. % for both the control and stressed tissue. Each measurement consisted of 9-12 separate determinations on 3 separate homogenates. The tests were replicated 4 times. The results obtained indicate that no large scale accumulation of alcohol occurs in the leaves. This does not, however, preclude the possibility that amounts may accumulate locally which are deleterious to membrane integrity and which are not detectable by this means. It should be added that this method also is sensitive for acetaldehyde, the postulated intermediate in ethanol fermentation.

With the above reservation, the results obtained from this spectrophotometric test indicate that very probably our earlier hypothesis suggesting that membrane damage resulted from alcohol fixation of constituent proteins may be invalid. Robertson (1959), Sjostrand (1963) and Thompson (1963), although differing on details of symmetry have postulated a membrane structure consisting of a bimolecular lipid layer surrounded on both sides by a structural protein monomolecular layer or perhaps in some cases by a polysaccharide

monomolecular layer. In any event, all investigators agree that in the lipid layer the molecules are oriented at right angles to the surface of the membrane with the hydrophobic ends of the molecules pointed towards the center of the membrane.

Current theories of cytological fixation of ultrastructural membranes (Wigglesworth, 1957; Criegee, 1936) agree that this property depends on the presence of unsaturated fatty acids within the membrane substructure. The recent work of Stoeckenius (1962) tends to validate this view.

The observations reported upon in the previous semi-annual report on this project indicate that rupture occurs in the chloroplast outer boundary membranes as a result of "blebs" or swellings which effectively separate the two surface protein layers of each unit membrane. The swellings may be either colloidal or osmotic phenomena, but, in any event, they apparently result from a degradation of the integrity of the bimolecular lipid layer. Such a loss of integrity would occur from a conversion of the hydrophobic ends of the lipid molecules to hydrophilic termini. Such a conversion in turn might be the consequence of the union of a low molecular weight organic acid with the lipid molecule at double bond position. This union, if the carboxyl group of the organic acid remained free, would result in the conversion of hydrophobic to hydrophilic groups, with the resultant imbibition of water. Barker and El Saifi (1952) have shown that potato tubers placed in a nitrogen atmosphere produce lactic acid in large amounts with a peak occurring after 10 days, following this, alcohol production subsequently increases and takes the place of a decreased lactic acid formation. According to Schneider (1960)

lactic acid formation under anaerobiosis has been demonstrated in sugar beets, carrots, germinating seeds, and the leaves of Rubus. Consequently, it appears possible that lactic acid might play a significant role in the ultrastructural events under consideration here, although it is conceivable that other low molecular weight organic acids involved either in glycolysis or the tricarboxylic acid cycle also play a part.

From the point of view of this theory, alcoholic fermentation may be viewed as a device which enables the plant to endure anaerobic conditions for a longer period of time since it results in the accumulation of a readily diffusible compound which, mole for mole, may be less deleterious to ultrastructural membrane structure than lactic acid or some other similar organic acid.

E. Tissue Dehydration Studies

The earliest comprehensive study which attempted to relate respiration rate to tissue water content in plant parts other than germinating seeds is that of Smith (1915). Smith measured the respiration rate in snowdrop leaves and stems of Tropaeolum and asparagus under various degrees of water loss. As the water content dropped from 100% to 75% on a fresh weight basis the respiration rate was observed to increase. A decrease in respiration was observed to occur only when the water content dropped below 50%. Iljin (1957) in a recent review agrees that, in general, water loss is accompanied by an increased respiration rate, at least, until relatively severe dessication is attained. Xerophytes, however, appear to be an exception, since they show little or no change in respiration following a water loss of 20 to 52%.

Singh and Varapande (1930), however, reported that a deficiency of water in leaves lowered their respiration rate.

In view of the apparent effect of tissue hydration on respiration rate and the relationship of this rate to survival of plants under low pressure it seemed desirable to investigate the ability of wilted oat plants to survive exposure to low pressure and relate this to the intensity of respiration under these conditions. In an experiment with greenhouse grown two-week-old oat seedlings, it was found that fully turgid plants completely succumbed in 8 1/4 hrs. under exposure to near vacuum while severely wilted plants exposed for the same length of time all survived, showing only moderate damage to the basal leaves and the tips of the younger leaves. Replication of this experiment gave the same striking results. The leaves of the turgid plants possessed approximately 90% water on a fresh weight basis, while the severely wilted plants had about 72% water.

Warburg manometric measurements conducted at various levels of tissue hydration correlated well with these results. The accompanying graph summarizes these measurements (fig. 8). It appears that in oat seedlings grown under these conditions that total leaf tissue respiration shows a decline with tissue dehydration. The increased ability of wilted plants to survive exposure to a near vacuum thus appears to be due to this decreased respiration rate. One might assume from these results that the glycolytic portion of respiration as well as the tricarboxylic acid cycle and the cytochrome system are interfered with by tissue dehydration. While it is not possible to infer at this juncture which of the segments of respiration is most susceptible to

tissue dehydration, it does seem logical to conclude that the increased survival ability of wilted plants is due to repression of a fermentative sequence responsible for the production of toxic products under anaerobic conditions.

II. Alpine Plant Experiments

As explained in the previous semi-annual report, a living collection of alpine plants from various locations was assembled during the summer of 1964. These were allowed to become established and testing was initiated during the winter and spring months. The plants in each case were exposed for varying lengths of time to near vacuum at the v.p. level of water (17+ mm. Hg. at 20°C) The following tables summarize the results of these experiments. In general, it appears that among those alpines tested, thicker stems, rhizomes, and portions of the root system are the most resistant parts of the plant to the effects of low pressure. Although most species appeared quite dead after two to four days stress under near vacuum conditions, at least partial recovery occurred in all cases, with one exception. This was the 96 hr. stressed plant of Cotoneaster congesta glacialis, which apparently was completely killed by the exposure. A grass, Koeleria crista, which was tested, showed a peculiar blooming pattern. Inflorescences developed on both control and experimental subjects after exposure, but seed failed to set on the stressed plants and the flowering culms rapidly died. The plant stressed for 96 hrs. produced only a few feeble flowering culms. Partial leaf recovery seems to have occurred in the experimental subjects of Saxifraga austromontana. This observation, if valid, would be remarkable since leaf tissue in all other subjects, once destroyed, seems to be incapable of recovery.

The previous semi-annual report noted the studies on Pentstemon procerus which we have conducted and the pattern of ultrastructural degradation which

Alpine Plant Experiments

Plant	Exposure Time	Date 1965	Immediate Results	Remarks
Ranunculus cymbalaria	6 hr.	2/23	no change.	After 4 months-all plants surviving; only 6 hr, plant has bloomed and produced stolons.
	9 "		some leaves, purplish discoloration at tips.	
Mt.Rose, Nev.	12 "		most leaves, purplish discoloration at tips.	
10,800 ft.	24 "		most leaves purplish, leaf margins curled.	
Sedum stenopetalum	6 hr.	3/2	no change.	All plants survived including the 48 hr. plant. Water soaked leaves turned black and excised.
	9 "		" "	
	12 "		" "	
Bullionville	24 "		" "	
Nev.	48 "		guttation at leaf tips; water soaked	After 4 months- all survived; bloomed-6,24 hr.Cont!
9,000 ft.			appearance in some leaves.	
Polemonium montrosense	6 hr.	3/9	no change.	Within 48 hr. the older leaves on the 12 hr. plant and all leaves on the 24 and 48 hr. plants were dead.
	9 "		" "	
	12 "		" "	
Mt. Rose	24 "		" "	
10,800 ft.	48 "		all leaves turned yellow-green.	6/29/65-all survived & bloom.
Koeleria crista	12 hr.	3/16	no change.	Within two days most of the leaves on the 96hr. plant had turned brown, few young leaves still green.
	24 "		normal guttation at leaf tips.	
Bullionville	48 "		" " " " "	
Nev.	96 "		guttation consisting of yellow droplets,	6/29/65- all survived(pg.13)
9,000 ft.			leaves bluish-black in color.	
Saxifraga austromontana	12 hr.	3/23	no change.	Within 1 day the 48 hr. plant developed red color at the leaf tips. Within 2 days the 72 hr. plant developed purplish black color at the leaf tips.
	24 "		" "	
	48 "		outer and older leaves turned yellow-green and water-soaked.	
Selkirks	72 "		most leaves water-soaked, yellow -green, and dull.	6/29/65-all survived;some affected leaves still alive but yellow; terminal buds injured,growth from lateral buds.
6,000 ft.				

Alpine Plant Experiments (cont.)

Plant	Exposure Time	Date 1965	Immediate Results	Remarks
Salix petrophila Winnemucaa Lake, Cal. 9,800 ft.	12 hr. 24 " 48 " 72 " 96 "	3/30	slight darkening of leaf tips. leaf tips slightly darkened, guttation norm. active guttation, leaves water-soaked. same as 48 hr. plant. leaves water soaked, curled at margins	12 hr. plant showed margin curling within 12 hr.;within 2 days all leaves are dead on the 48,72, and96 hr. plants, buds still green. 6/29/65-all survived & growing well.
Cotoneaster congesta glacialis obtained commercially	12 hr. 24 hr 48 " 96 "	4/5	no change. leaf tips darkened. all leaves affected, water-soaked, brownish. same as 48 hr. plant.	Within 12 hr. some leaves on 12 hr. plant turned brown. All leaves turned brown on 48 and 96 hr. plants. 6/29/65- 96 hr. plant dead; 48hr.-top dead,shoots alive.
Carex sp. White Mt. Cal. 13,200 ft.	12 hr. 24 " 48 " 72 " 96 "	4/27	no change. " " " " in color, guttation at leaf tips. slight water-soaked appearance at leaf tips. leaf blades turned a dull yellow.	All plants eventually recovered, but 72 & 96 hr. plants remain stunted after two months.
Anemone globosa Beartooth Mts. 11,000 ft.	24 hr. 48 " 60 " 72 "	5/3	leaves brown. leaves brown; green under bell jar but turn immediately on exposure to air,-indicates polyphenoloxidase. same as 48 hr. plant. same as 48 hr. plant.	After two months-all plants survived;severely damaged plants sent up new leaves; only 24 hr. & control bloomed.
Trifolium nanum Mt. Evans, Colo. 14,000+ft.	12 hr. 24 " 48 " 72 " 96 "	5/10	leaf tips and occasionally entire leaf water-soaked. nearly all leaves water-soaked. leaves yellow and water-soaked. leaves yellow-brown, water soaked. leaves brown, water soaked, small white spots.	Within 4 days most leaves on the 12 and 24 hr. plants were dead. Within two days all leaves on the 48,72,& 96 hr. plants were dead. 6/29/65= all plants survived; 72 & 96 hr. plants remain stunted.

resembles that of Avena sativa.

It is the intention to continue these studies on alpine plants and to complement the macroscopic stress observations with electron microscope examinations where this seems warranted.

III. Papers

A. Prepared:

"Reversible and Irreversible Changes Induced in Chloroplasts by Reduced Pressure." (abs.).- Twenty-second Annual Meeting - Electron Microscopy Soc. of America. (Oct. 1964).

"Effect of Reduced Barometric Pressure on Plants." - for "Environmental Biology" - Federation of American Societies for Experimental Biology (Feb. 1965).

"Physiological Studies of Avena sativa and Ultrastructural Observations on Chloroplasts under Conditions of Reduced Pressure - Master's Dissertation by Richard N. Trelease (Research Assistant). (May 1965)

B. In Preparation:

1. Ultrastructural changes induced in Avena chloroplasts by exposure to low pressure.
2. Tissue hydration and survival under low pressure.
3. Seed germination under low pressure and ecological considerations.
4. Avena survival under near vacuum as influenced by thermoperiod.

IV. Research Continuation.

It is anticipated that the thermoperiod, hydroponic, and alpine plant experiments will be continued. An attempt will be made to gain additional supporting evidence for the theory presented in this report. Membrane degradation theory will be tested by the construction of an artificial lipid-protein membrane system and the subjection of this system to various reagents which might be expected to combine with a lipid bimolecular layer in the manner postulated.

It is anticipated that labelling with $C^{14}O_2$ will be used and combined with thin-layer chromatography techniques in an attempt to identify the organic acids which accumulate in the Avena plant under near vacuum conditions and to define the intermediary metabolic sequence contributing to the observed membrane breakdown.

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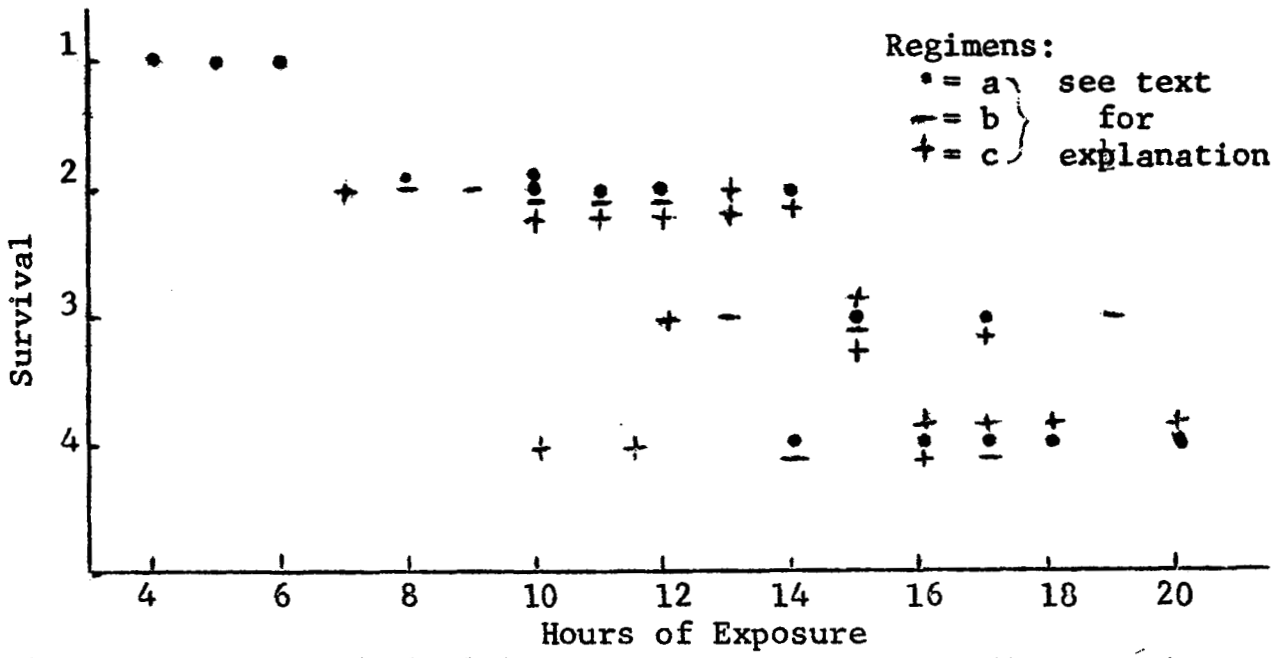

Fig. 1. Survival of chamber-grown Avena sativa seedlings under various light and temperature regimens. CODE: Survival values,- 1=Complete survival, no macroscopic damage; 2=Some discoloration of leaf tips, at least 3/4ths of the plants in each pot remaining alive; 3=Considerable leaf ~~dest~~ destruction, less than 3/4ths of the plants in each pot remaining alive; 4=Complete destruction of all plants.

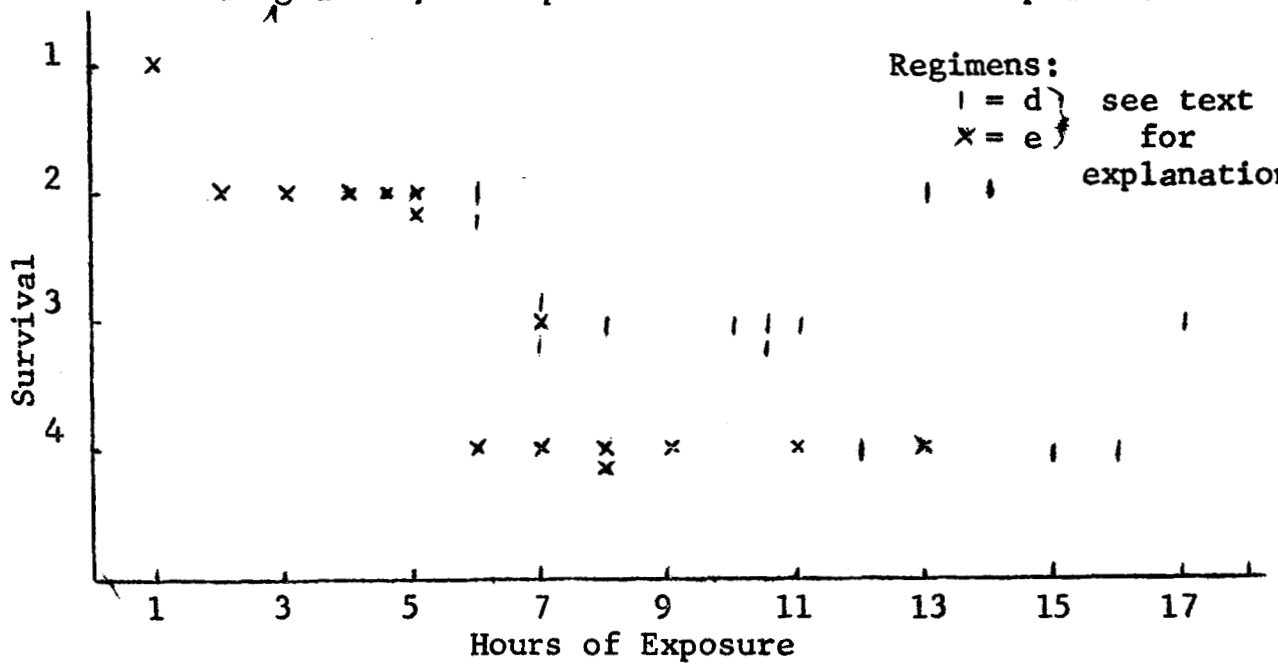

Fig. 2. Survival of chamber-grown Avena sativa seedlings under various light and temperature regimens. CODE: Survival values, - Same as Fig. 1.

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Figure 3 - Oat seedlings grown in a complete Hoagland's medium 15 days after exposure to near vacuum for varying lengths of time. From left to right the periods of exposure were: 8 hr; 7 hr; 6 hr.; 5 hr.; control,- no exposure; control - no exposure.

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Fig. 4 - Oat seedlings grown in a -N Hoagland's medium 22 days after exposure to near vacuum. From left to right the periods of exposure were: 8 hr.; 7 hr.; 6 hr.; 5 hr.; control (-N) no exposure; control (complete) no exposure.

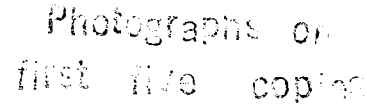

Fig. 5 Oat seedlings grown in a -P Hoagland's medium 43 days after exposure to near vacuum. From left to right the periods of exposure were: Complete (no exposure); -P, no exposure; 5 hr.; 6 hr.; 7 hr.; 8 hr.

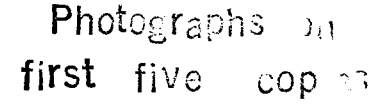

Fig. 6. Oat seedlings grown in a -S Hoagland's medium 32 days after exposure to near vacuum. From left to right the periods of exposure were: 8 hr.; 7 hr.; 6 hr.; 5 hr.; -S no exposure; complete no exposure.

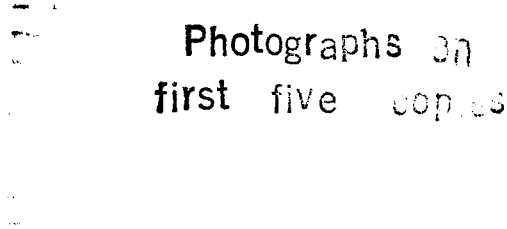

Fig. 7. Oat seedlings grown in various media and all exposed to 5 1/2 hr. of near vacuum as they appeared 50 days after exposure. From left to right: complete; -S; -P; -N.

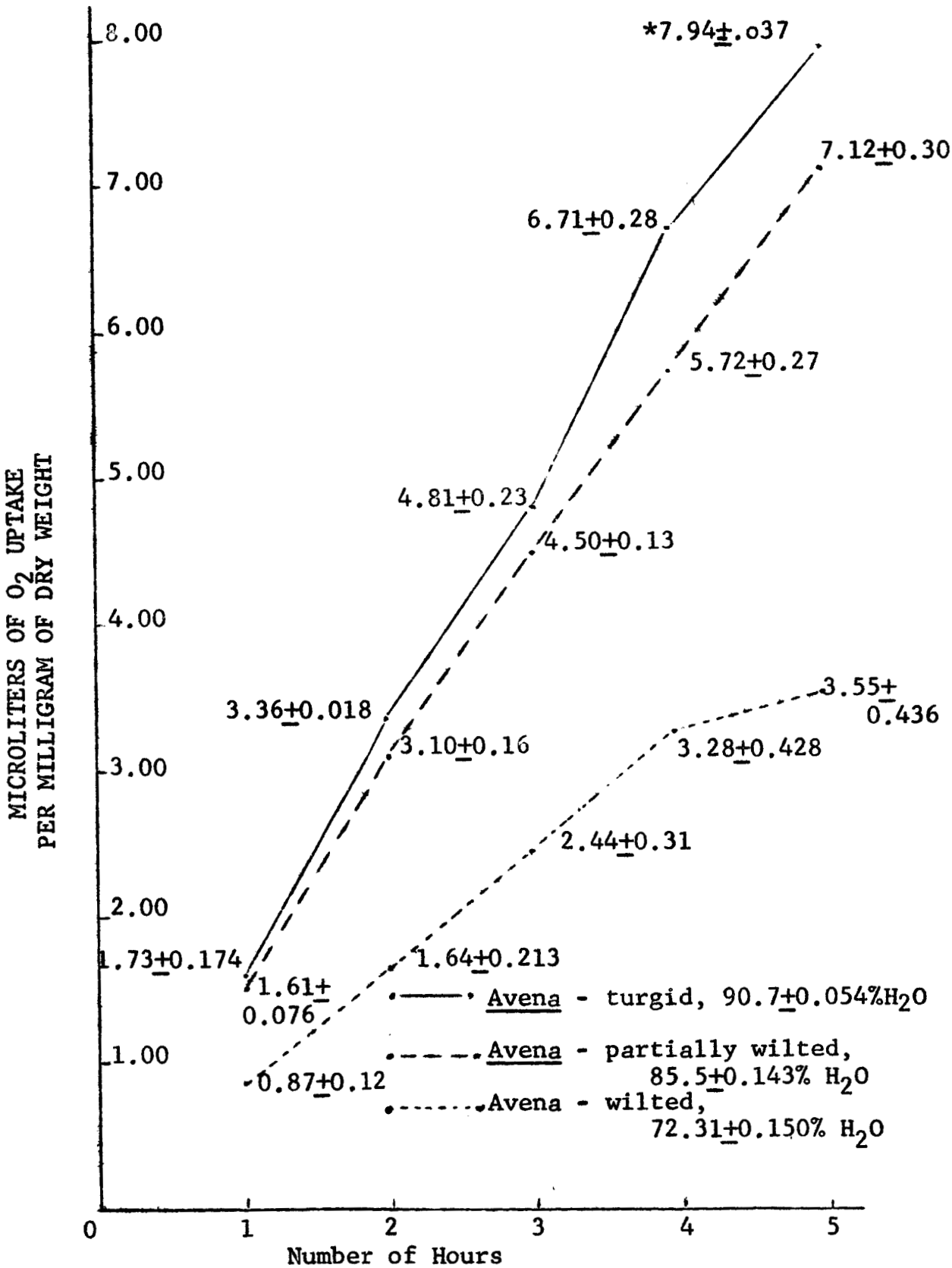

Fig. 8. Cumulative oxygen uptake of Avena in various stages of hydration over a five hour period.
*Mean microliters oxygen uptake ± standard error.